'Immunocytochemical Localization and Biochemical Estimation of Peptide Hormone Binding Capacity of Breast Tissue'

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CERTIFICATE

This is to certify that the study entitled **"IMMUNOCYTO-CHEMICAL LOCALIZATION AND BIOCHEMICAL ESTIMATION OF PEPTIDE HORMONE BINDING CAPACITY OF BREAST TISSUE"** *has been carried out by* **Sarita Tandon** *under my supervision and guidance.*

She has fulfilled the requirements of the Aligarh Muslim University for the degree of Master of Philosophy in Bio-chemistry.

The work included in this thesis is original unless stated otherwise and has not been submitted for any other degree.

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Her method, observation and results were checked by us from time to time.

The work included in this thesis is original unless stated otherwise and has not been submitted for any other degree.

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CONTENTS

ABBREVATIONS:

Ab	:	Antibody
Ag	:	Antigen
BBD	:	Benign Breast Disease
DA	:	Dopamine
ER	:	Oestrogen Receptor
HRP	:	Horse Radish Peroxidase
PAP	:	Peroxidase Anti Peroxidase
PBS	:	Phosphate Buffered Saline
PR	:	Progesterone receptor
PRL	:	Prolactin
PRL-R	:	Prolactin Receptor
TSH	:	Thyroid Stimulating Hormone

INTRODUCTION

INTRODUCTION

There is a general agreement that the breast is hormonally controlled, however few data exist regarding the mechanism of action and interaction of these hormones and the responses of breast tissue. Breast may also be a primary endocrine organ as suggested by Diamond (1982). It was seen that microarchitecture pattern of breast tissue changes in response to hormonal changes throughout the menstrual cycle and during the life of the women (Vogel et al 1981). Recent evidence suggests that a neuroendocrine imbalance is likely cause of benign breast disorders. However studies of both steroids and peptide hormones have shown conflicting results and no precise hormonal abnormalities has emerged so far (Kumar et al 1984).

Jick et al (1974) suggested that the clinical use of dopamine antagonists such as the Rauwolfia drugs and the Phenothazines, which leads to an elevation of (PRL) secretion, resulted in an increased incidence of breast tumours, but this study was contradicted by other (Mack et al 1975, Wagner 1978).

Later on the involvement of PRL in sustaining the growth of experimentally induced rat mammary tumours was established (Partridge et al 1979). A number of studies of plasma PRL level in patients with breast cancer and controls have been performed but the results present a mixed picture (Kwa et al 1974, Wang et al 1987), may be because of the pulsatile secretion of the hormone.

The importance of PRL receptors as an index of responsiveness of the tumour tissue has been established in recent years as hormones influence cells by first binding to high affinity receptor protein located either on the cell surface or in the cell interior. Various studies have been done to locate the PRL receptors in benign and malignant tissue of breast. Evaluation of the activity of hormone receptors as an index of response of treatment with either hormone administration or endocrine ablation is clearly of clinical importance.

This study was designed to examine immunohistochemically detectable PRL in various breast lesions using a highly sensitive modified version of Dinitro-phenyl (DNP) hapten sandwich staining (DHSS) procedure (Jasani et al 1981) Efforts are in progress for biochemical estimation of PRL binding in particulate membrane preparation of breast tissue. The other peptide hormones of anterior pituitary like LH & FSH which have a direct trophic action on gonads may also be having a direct relationship to breast. Attempts are being made to study the binding of these on breast tissue preparations.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 ENDOCRINE CONTROL OF BREAST:

Although there is a general agreement that the breast is hormonally controlled it is not yet known whether human breast is purely endocrine target organ or itself secretes hormones into the blood, (Kolodny et al 1972). The in vivo hormonal studies indicate that mammary tissue regresses following removal of the specific endocrine gland, and that the growth can be restored and even stimulated in these regressed tissue by supplying the hosts with specific hormones from the same endocrine glands. Also none of the hormones, alone or in combination, have shown consistently to be growth promoting in vitro for unprimed or regressed mammary tissue.

The major development in the female breast occurs at the time of puberty which is characterized by a number of endocrine changes (Styne & Grumbach 1978). After puberty owing to the cycle production of oestrogen by the ovaries, accelerated rate of mammary growth take place. Oestrogen is the hormone responsible for development of the ductal epithelium whereas progesterone is associated with lobular alveolar proliferation. Progesterone may also act as an antiestrogen, antagonizing the action of oestradiol on duct cells (Vorherr and Messer, 1978) by decreasing the concentration of oestrogen receptors (Kuttenn, et al 1981). A ratio of oestrogen to progesterone of 1:20 to 1:100 is considered to be optimal for mammary development.

PRL together with the sex steroids promotes growth of the ductulolobular alveolar structure. In addition PRL is considered to be the significant lactogenic hormone. The endocrine control of milk formation by the differential breast is complex requiring in addition to appropriate primary by oestrogen and progesterone, specific lactogenic hormones and the permissive action of glucocorticoid insulin, thyroxine, and in some species growth hormones. Thus as endocrine target organ the breast has very complex control mechanism (Diamond 1982).

2.2 PROLACTIN:

Ovine PRL has been isolated in highly purified form by various investigators (white et al 1937, 1942, Li et al 1940a, 1941, Cole and Li 1955). It is a protein of molecular weight 23,300 (Li et al 1957) and isoelectric point at PH5.7. It consists of a single polypeptide chain (Li 1957) with two tryptophan residues and three disulphide bridges (Li 1949), Ovine PRL consists of 198 amino acids with threonine at the amino terminus and half cysteine at the carboxyl end. Thus it has free amino acid group but no free carboxyl terminus. For this reason, Li (1949) suggested that the peptide chain has an intrachain disulphide bridge forming a ring. The three disulphide bridges are found between residues 4 and 11, between residues 190 and 198 and between 58 and 173. The two tryptophan residues are in positions 90 and 149 and seven metheonine residues are in position 24, 36, 53, 81, 104, 129 and 131. There are seven

tyrosine residues in PRL. One of these tyrosine residues is buried as revealed by spectorophotometric titration of the hormones in KCL solution (Ma et al 1970). The buried tyrosine becomes ionized only after extensive alkali denaturation. However, all seven tyrosyl residues were found to react with tetranitromethane (Ma et al 1970).

Spectrophotometric titrations and rates of tryptic digestion indicated that nitration of the molecule produced significant conformational changes in it. Apparently the biological and immunological properties of PRL do not depend upon the intergrity of tyrosine residues as well as the molecular conformation. No bound carbohydrate has been found in PRL.

Procedures which are satisfactory for the isolation of PRL from the pituitaries of other species have not proved effective with human pituitaries. While fractions possessing PRL activity have been obtained, these fractions have all been rich in growth hormones. Thus the nature of PRL remains a mystery of current pituitary endocrinology.

PRL is found to have many physiological roles in animals. It has a luteotrophic action on the ovary in some species, it induces changes in maternal behaviour which are important for the helpless young, and it has general metabolic actions in the hypophysectomized animals which are unrelated to reproduction. Also under suitable experimental conditions PRL has been shown

to be calorigenic and diabetogenic as well as to promote protein synthesis and to increase the rate of choridroitin sulfate formation in cartilage. In most species the PRL content of pituitaries from female animals higher then that of male animals. PRL, a peptide hormone synthesized in the pituitary, plays the critical role in the initiation as well as maintenance of milk secretion if sukling stimulus is present (Noel et al 1974).

2.3 CONTROL OF PROLACTIN SECRETION:

PRL differs from other anterior pituitary hormones in that its secretion is under predominant toxic inhibition by the hypothalamus. Thus any interference, be it surgical or due to disease which disrupts hypothalamic/pituitary connections results in hyperprolactinaemia. There is a considerable body of data supporting the hypothesis that dopamine (DA) is the natural PRL inhibitor factor. The concentration 10^{-9} to 10^{-7} mole/1 of DA is required on to the portal capillary bed to inhibit the release of PRL from the anterior pituitary gland. Furthermore, drugs which inhibits the release of DA into portal blood result in enhanced PRL release, whereas therapeutic agents stimulating DA secretion into portal blood results in suppression of PRL secretion.

In man TRH is a potent stimulator of PRL release. Tashjian et al (1971) showed that TRH stimulate PRL release in vitro. Basal PRL level fall following dilivery, but PRL secretion is

enhanced by stimulation of breast such as the act of nursing (the so called suckling relex) which requires the presence of other hormone, oxytocin.

2.4 ROLE OF HORMONES IN MAMMARY TUMORIGENESIS:

Breast undergoes changes throughout the menstrual cycle and during the life of women and thus is affected by various hormones like oestrogen, progesterone, androgen, growth hormone, prolactin, insulin, corticosteroids, gluco-corticoids and thyroxine. The reproductive endocrine system which changes during the menarche, the menopause and after child birth may contribute considerably to the aetiology and pathogenesis of breast cancer. Mammary cancer is not a homogenous entity but it is a heterogenous collection of carcinoma of varying biologic potentials and only a few consistent hormonal relationships have emerged. There is an increase cancer risk with early menarche and late menopause. Oophorectomy early in life exerts a protective effect against mammary carcinoma as does an early full term pregnancy. In some cases there is regression of tumour when treated with oestrogen. This establised an idea that there may be a significant endocrine abnormality in women with breast cancer.

Sherman et al (1974) put forth a hypothesis attempting to provide a pathophysiological interpretation of the various endocrine related risk factors of breast diseases as shown in

table-1. They further indicate that some of these risk factors interact and are not necessarily independent of each other.

Breast cancer risk factors (Sherman et al 1974) (TABLE-1)

Increased	Decreased	No effect
Late first pregnancy Nulliparity, Early menarche, Late menopause, obesity, low androgen excretion.	Early first pregnancy, castration	Oral contraceptive, Lactation, Parity

Many of the first situation might be associated with abnormal ovarian follicular maturation as well as irregular or dificient progesterone production by coprus luteum. This would result in a hormonal environment of relative oestrogen excess and progesterone deficiency, a situation theoretically related to the development and progagation of breast neoplasia. They hypothesis was based on several assumptions and observations.

(i) Oestrogen, if not itself an inducer of breast cancer are important cofactors in the etiology of breast cancer (Mac Mohan et al 1973, Cole et al 1978, Nisker and Siiteri 1981).

(ii) Progesterone is an important inhibitor of oestrogen action (Botella-Llusia 1973, Nisker and Siiteri 1981) though it also synergizes with the latter to produce the differentiated cell population in the breast.

(iii) Corpus luteum progesterone production is a common consequence of abnormal follicular maturation. It may be manifested as limited progesterone secretion during a luteal

phase of either normal (inadequate luteal phase or diminished (Short luteal phase) length.

(iv) Prior to the menopause, there is a variable period of time during which menstural cycles are irregular in most women. (Treloar et al 1967). At this time a period of unopposed oestrogenic stimulation of up to 8 years may occur. He thus proposed that late menopause might be associated with a greater proportions of cycles exhibiting progesterone deficiency.

(v) Like early menarche, a breast cancer risk factor, might be associated with an earlier onset of and longer exposure to an environment characterised by oestrogen production in the absence of the regular secretion of appropriate amounts of progesterone (Mac Mohan et al 1982b).

(vi) Age at first full term pregnancy is strong determinant of the breast cancer risk, the earlier the first birth the less is the risk (Wynder et al 1960, Mac Mohan et al 1970, 1973, Craig et al, 1974, Henderson et al, 1974), Drife (1981) has proposed that it occurs because of differential responsiveness of breast tissue before and after the first full term pregnancy to the protective effects of progesterone. He explained this as before a first full term pregnancy the breast has few progesterone receptors, and therefore, during the normal menstrual cycles the breast is stimulated largely by oestrogens. Further, he

suggested that a first full term pregnancy somehow results in the development of progesterone receptors so that after the first birth, normal cycles result in adequate stimulation of the mammary ductal cells by progesterone cellular differentiation and a consequent reduction in risk of breast cancer. The exact role of reproductive endocrine system in the development of mammary cancer remains to be clarified.

2.5. HUMAN PROLACTIN IN BENIGN BREAST DISEASE:

Several reports on plasma PRL levels in patients with various benign breast diseases have been based on single samples and the possibility of abnormal profile due to mentioned stages and emotional and other conditions can not be excluded. In order to avoid these influences, PRL levels have been estimated in serum samples taken daily through out the menstural cycle of patients with benign breast disease, women who suffer cyclical mastalgia frequently show a slight elevation of serum PRL (Cole et al, 1977), Moreover, a significant positive correlation was present between age and PRL in cystic breast disease (Cole et al 1977) but not in mammary fibroadenosis or normal women. It was also found that symptoms of mastalgia can be relieved by inhibiting PRL secretion with the dopamine agonist bromocriptine (Mansel et al 1978). This result was supported by Kumar et al (1984) in which it was found that release of PRL, LH and FSH was significantly greater in cyclical mastalgia patients than controls and suggested an alteration in lactotroph in these patients.

2.6. HUMAN PROLACTIN IN BREAST CANCER:

Higher plasma PRL levels have been reported in breast cancer patients than in healthy controls (Murray et al 1972, Rolendi, et al 1974) but this could not be confirmed by other studies (Frank et al 1974, Dicky 1972, Boyns, 1973), Sarfaty et al (1976) compared plasma PRL levels in women and in patients with primary or metastatic breast cancer. Whereas PRL levels were generally higher in normal premenopausal women or breast cancer patients than in respective post menopausal subjects or in women after ovariectomy, within each category breast cancer patients had significantly increased PRL values. Following ovariectomy, PRL levels dropped more sharply in responders than in non responders to endocrine therapy (Sarfaty et al 1976).

It has been reported that pituitary PRL varies according to age, menstrual stage, drug usage, etc., also it fluctuates by the time of day and changes in response to emotional conditions. Since hormonal differences may become blunted with increasing age and/or debelety, increased PRL secretion in older women with breast cancer for instance may be marked by normal old age related decline of PRL secretion. According to Robyn (1975) PRL may favour both development and progression of breast carcinoma but evidence for that is still indirect and rather speculative while it has been proposed, even if endogenous PRL by itself may not be an etiologic factor, it would in conjunction with sex steroids contribute to an accelerated malignant

mammary growth as indicated in the more fulminant course of breast cancer during pregnancy. PRL is known to exert several physiologic actions on breast cells (Shiu and Friesen 1980). PRL regulates water and electrolyte balance, milk protein synthesis, uridine conversion and incorporation into DNA, and breast fatty acid synthetase activity. An increased synthesis of oestrogen receptor has also been reported. Kim and Furth (1977) stated emphatically that all mammary cancer risk factors described for human subjects and animals points to a role of PRL in mammary carcinogenesis albiet it has been suggested that PRL, if associated with breast cancer, must do so at normal levels (Wilson 1973).

2.7. HORMONES RECEPTORS:

The biological effect of hormones is dependent on at least two factors, serum concentration of hormones and target tissue responsiveness. The protiens or glucoproteins located on or in target cells initially bind hormones and then translate the hormonal message into hormonal actions. Any change in hormone receptors are considered as one determinant of tissue responsiveness. Thus these receptors are not only important in carrying out physiological regulation of endocrine target cells, but are also the potential sites of disordered function of the endocrine system. Hormone receptors or binding sites have been demonstrated in various preparations of human and animal mammary tissue for oestrogens (Korenman & Dukes 1970, Gardner & Wittliff 1973, Hunt & Muldoon 1977) and progesterone (Mc Guire & Horwitz 1978).

Realising the importance of hormones and their effect on mammary tumourigenesis, attempts were made by various workers to study in detail the hormone profile in BBD patients and breast cancer patients, and estimation of hormone receptors and their valve have also helped in the treatment of breast disease especially in the case of steroid receptors (Calandra et al 1984).

PRL is one of the principal hormones regulating the alveolar function of the mammary gland (Anderson, R.R. 1974, Vorherr, H. 1974). PRL first binds to specific receptors located in the plasma membrane of the cell. This is the first even in the biological action of PRL and other polypeptide hormones on the target tissues. Following this binding a number of effects within the cell can be observed. These effects include at the nuclear level, an activation of the transcription of milk protein-genes and a stimulation of DNA synthesis. The occurance of a specific receptor for PRL was first reported in mouse mammary glands by Turkinton (1970). The significance of assay of PRL receptor as a quantitative index of the responsiveness of the target tissue has been emphasized as monitoring of the receptors on mammary tumour cells may have the potential of predicting the responses of the cells to prophylasis and therapeutic actions of PRL.

2.7(a) **OESTROGEN AND PROGESTERONE RECEPTORS IN HUMAN BREAST:**

The value of determining the presence of oestrogen receptor (ER) and progesterone receptor (PR) in predecting clinical response of breast cancer patients to hormone therapy is well recog-

nised, various biochemical procedures have been accepted for the routine detection of solubilised steroid receptor protein in human breast cancer. Histochemical and immunohistologic visualization of steroid hormone binding in human malignant breast tissue has also been attempted. (Louis et al, 1982). An immunohistochemical method utilizing polyclonal antibodies to cytoplasmic ER and precipitation of cytoplasm processing procedure which allows in situ precipitation of cytoplasm ER value were used. (Raam et al, 1987).

Both the quantitative ER levels and the percentage of ER positive specimen were directly related to the age of patient. As a result of this strong relationship between age and ER, specimen from postmenopausal patients had higher ER levels than specimen from premenopausal patients. But within individuals of the same age, the proportion with receptors appears to be unrelated to menopausal status (Thorpe et al, 1983) suggesting that age is a more important determinant of receptor status than menopause. However, Bertuzzi et al, (1981) found that the proliferative activity of tumours from patients with the same menopausal status was higher in ER negative tumours than in ER positive tumours. The proliferative activity decreases from premenopausal to postmenopause with both ER positive and ER negative tumours suggesting that there may be factors other than oestrogen inversly related to the patients age that

play a determinental role in cell proliferation of breast cancer. The relationship between steroid receptors and size of primary tumour reveals a tendency that large tumour to be ER negative (Holdaways and Mountjoy 1978). Large tumours might have evidence of tumour necrosis that could result in a lack of steroid receptors or they may simply be an indication of more aggressive disease.

It might the hypothesized that for patients with large tumours the receptor concentration would be higher for node positive patients than for those with negative nodes. However subsequent analysis failed to identify any correlation between nodal involvement and receptor concentration even for large tumours.

These appears to be no relationship with progesterone receptor for either age on menopausal status when these variable were analysed seperately. But premenopausal women had higher progesterone receptor concentration than postmenopausal women when patients of the same age were compound, perhaps reflecting greater estrogen- mediated synthesis of progesterone receptor. (Clark et al 1984).

2.7(b) **PROLACTIN RECEPTOR**:

The existence of PRL receptors (PRL-R) in many different animal species is supported by extensive evidence. Since more than 80 diverse biological activities have been reported for PRL, it is not surprising that PRL-Rs have been identified in

many different organs. In mammals, the mammary gland and the gonads are undoubtedly major target organs at which PRL expresses its biological actions. De-Souza et al (1976) visualized PRL-R with immunoperoxidase technique. Later on Purnell et al, (1982) and Chadly and Walker (1983) concluded that immunocytochemistry is a suitable method for demonstration of PRL binding sites in human breast tissue and provide useful information with regard to tumour heterogenesity.

Partridge and Hannel (1979) reported the examination of eight primary human breast carcinomas and one secondary human breast carcinoma (Scalp), which revealed evidence for the presence of PRL binding sites. Later on Peyrat et al, (1982), Carlo et al (1984) Hermite-Baleriaux, et al ... (1987) and various other workers have determined the PRL-R in human breast tumours membrane preparations.

2.7(c) RELATIONSHIP BETWEEN OESTROGEN, PROGESTERONE AND PROLACTIN RECEPTORS:

Some studies indicated that PRL and oestrogen are the two hormones implicated in the regulation of mammary gland growth. No correlation, has been shown between serum PRL levels and these steroid receptors values in breast cancer (Vinka et al 1980). Never the less, a recent publication (Ben David 1981) reported a higher dependency of breast cancer on PRL than on steroid receptor according to their study on binding sites PRL (PRL-R).

Calandra et al (1984) studied the receptors in breast with the aim.

1. To investigate the receptor status (ER Vs PR in primary and metastatic mammary carcinoma.
2. To correlate the existence of ER/PR with some clinical feature.
3. To assess the interrelationship between ER/PRL-R in some tumours.
4. To established the incidence of these receptors in the local population in comparison with what has been published by other groups (Mc Guire et al, 1978, Vinko et al, 1980). They have considered a positive ER(ER+) assay for human breast specimen when the number of binding site was equal to/on higher than 2 mol/mg protein and for PR (PR+) level equal to/or higher 5 mol/mg protein.

Concentration of oestrogen and progesterone receptors (ER PR in cytosol f mol/mg in the group and the comparison between pre & post menopausal patient with human breast carcinoma was given by them as below:-

TABLE-2

Whole group	ER	140.3 +/- 11.1*	(970)**
	PR	210.2 +/- 16.3	(970)
Premenopausal	ER	60.8 +/- 7.3	168
	PR	190.2 +/- 23.1	168

TABLE Contd.....

Postmenopausal	ER	176.2 +/- 19.6	802
	PR	203.4 +/- 19.6	802

* Mean +/- SEM.
** Number of patients investigated.

These results agree with the finding of another groups and particularly to the higher ER level in post menopausal patients. They also studied the incidence of breast tumours containing or not PRL-R and found that it is equally distributed (48% Vs. 52%) and there was a lack of correlation between PRL-R and ER. were approximately equally seen in the the presence or absence of PRL-R (34% Vs. 42%). Table-3.

Distribution of prolaction and oestrogen receptors (PRL-R, ER) in human breast carcinoma.

TABLE-3

PRL-R + ER +	17/50*	34%
PRL-R + ER -	7/50	14%
PRL-R - ER +	21/50	42%
PRL-R - ER -	5/50	10%

* Number of patients investigated.

2.8. IMMUNOCYTOCHEMICAL METHOD OF LOCALIZATION OF TISSUE ANTIGEN:

The possibility of locating specific substances that can

not be precisely characterised by special stains would appear to be a useful addition to the diagnostic armoury of the Pathologists. Immunocytochemistry has been available as an investigative tool since the pioneering work of Coons et al (1942) with fluorecein labelled antibody. As the name suggests it identifies a tissue antigen with reference to morphology by means of a specific antigen antibody reaction rendered visible by a suitable marker (fluorochrome). With this capacity, this technique has made many important contributions to the advancement of both medical and biological knowledge. Though immunofluorescent technique are the most widely used immunocytochemical method because of its speed and simplicity in routine laboratories, yet it is now being replaced by new techniques due to certain limitation it possesses. It could only be applied to fresh frozen unfixed tissue. Formaline paraffin processed material could not be used. Further, unstability of fluorescence phenomenon, requirement of expensive equipments, for its examination, difficulty in quantitation, lack of electron opacity made it necessary to use alternative marker. Avrameas & Uriel (1966) and Nakane and Pierce (1966) used enzyme as immunochemical markers to over come many of the limitation of fluorescent methods. Further several antigens of histological importance, including intracellular immunoglobulins and hepatitis B antigen were demonstrated in formaline paraffin sections by immunoperoxidase method (Burns, et al 1974, 1975 a,b,c). As the name immunohistochemistry suggests, the technique is a combination of immunocytochemestry which

attaches the tracer or marker to the specific antigen within the tissue sections, and standard enzyme histochemistry which visualysis the tracer for bright field or electron microscopy. Any enzyme that can be detected by a reliable histochemical method and that does not modify the structure of the cells, can be employed. Most commonly used enzymes are peroxidase, glucose oxidase, acid or alkaline phosphatase. Horse raddish peroxidise is most commonly used. They have high specific activity and are relatively stable at room temperature. The antibody is labelled with suitable enzyme and the labelled antibody antigen complex was revealed by ordinary light microscopy by treating it with 3-3 diaminobenzidine tetrahydrochloride (DAB) and hydrogen peroxide (H_2O_2) (Graham & Karnovsky 1966). The brown colour developed at the site of enzyme activity was stable and could be rendered electron opaque with osmium tetraoxide (O_sO_4). Thus many of the disadvantages of immunofluorescence were removed by using enzyme as tracer. Using this principle different method could be followed.

Direct Method: (Fig.1a)

In this, the primary antiserum directed against a particular antigen is covalently linked to horse raddish peroxidase by chemical conjugation. This HRP reacts with the DAB substrate giving a brown colour. Here larger amount of antisera is required antibody is directly conjugated with enzyme and often the yield is less. (Modesto and Pesce 1971). This is the least sensitive method.

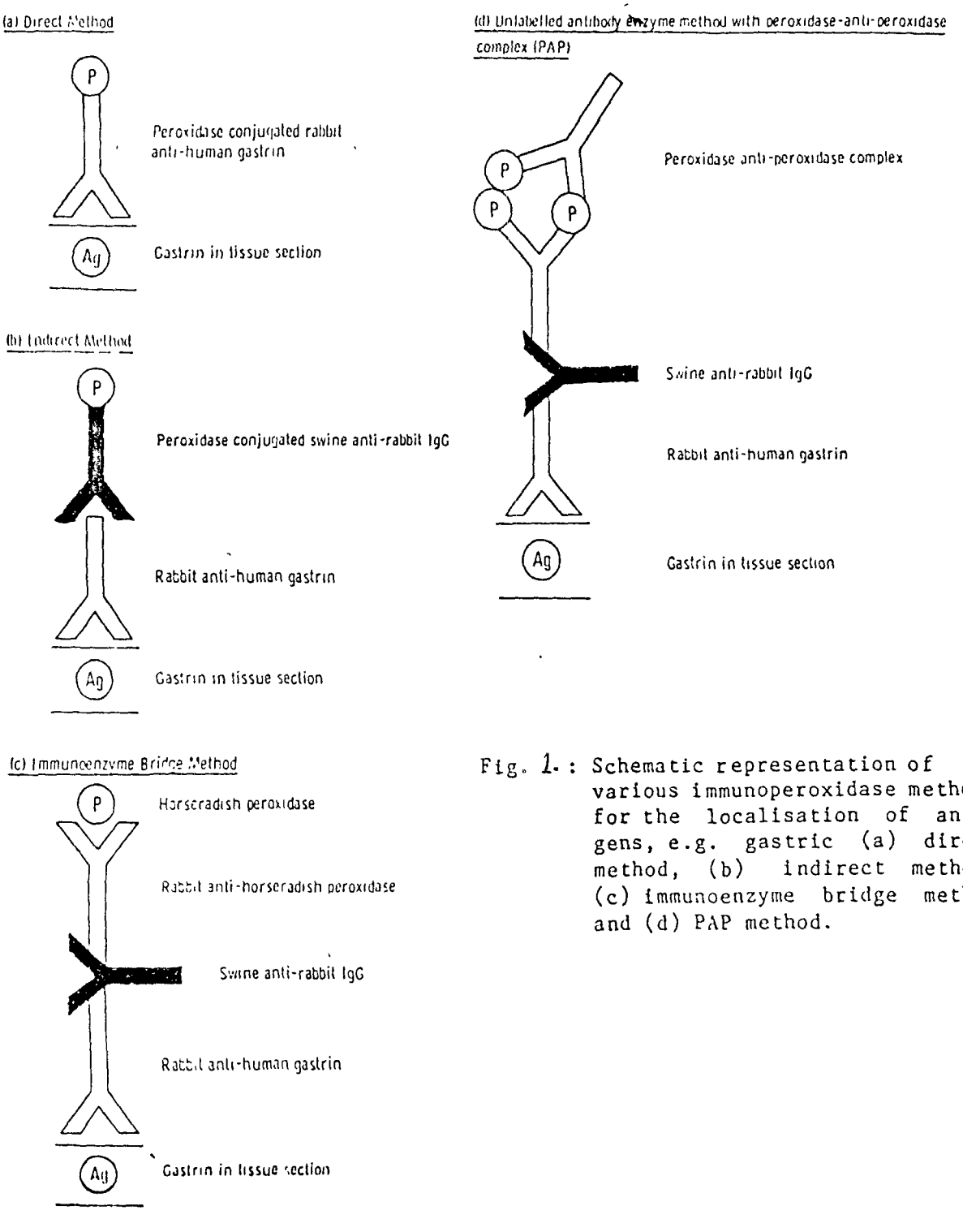

Fig. 1.: Schematic representation of the various immunoperoxidase methods for the localisation of antigens, e.g. gastric (a) direct method, (b) indirect method, (c) immunoenzyme bridge method and (d) PAP method.

Indirect Method: (Fig.1b)

In the indirect method, antigen in tissue section is first reacted with its unlabelled antibody (e.g. rabbit anti human). This antigen antibody complex is then reacted with the second antibody which is directed against immunoglobulin of the species in which the first antibody is raised. The 2nd antibody is labelled with enzyme (Swine antirabbit IgG), and the colour is developed with DAB. In this method the sensitivity is increased. Also less amount of primary antisera is required and purified conjugated antisera against Ig of several species are readily available.

Immunoenzyme Bridge Method: (Fig.1c)

The immunoenzyme bridge method was introduced by Manson (1969). In this method antigen specific primary antibody, raised in rabbit, is applied to the section. In the second step, swine anti-rabbit IgG is applied in excess. Next antiperoxidase serum, raised in rabbit IgG molecule, it will bind to the free site of the swine antirabbit IgG antibody. Then the section is incubated in a solution containing horseraddish peroxidase and the enzyme attached to the specific conbining sites on the antiperoxidase antibody. Finally, the bound peroxidase is visualised by the reaction of DAB. This method rapidly superceded the techniques employing conjugated antisera because in addition to low yield, conjugation also produces large aggregates of antibody molecule which, if attached to peroxidase causes high background staining.

Unlabelled antibody enzyme method using PAP complex: (Fig.1d)

To over come the inherent difficulty of the above method Sternberger et al (1970) introduced the use of a soluble peroxidase antiperoxidase complex (PAP) as an enzyme. This stable cyclical complex is formed by reacting horse raddish peroxidase and hyperimmune rabbit sera containing anti-peroxidase antibodies at low pH and withmoderate antigen excess. The first two steps of this method are identical to those of the immunoenzyme bridge method i.e. rabbit antigen serum followed by excess swine anti-rabbit IgG. In the third step PAP complex is applied. The peroxidase component of the complex is then visualised by treating it with DAB. This method is more sensitive as well as shorter then immunoenzyme bridge method. Further refinements of these methods have been made by various workers.

The two factors which may interfere with the interpretations of the result of the immunoperoxidase techniques, are (i) endogenous peroxidase activity, and back-ground staining.

These particularly occur in formalin paraffin sections.

1. **Endogenous Peroxidase Activity:**

This activity occurs in red blood cells, granulocytes (especially eosinophils) and acid haematin. It can be dealt with by either of the following methods:

(a) It could be prestained in a colour other than that of the immunoperoxidase stained antigen complex (Robinson and Dawson 1975).

(b) It could be inhibited or removed prior to immunoperoxidase staining by treating the tissue sections with 0.5% H_2O_2 in methanol for 30 minutes (Burns, 1975b) or 3.0% H_2O_2 in water for 3 to 10 minutes (Garvin, Spicer et al 1976) also use of periodic acid followed by sodium borohydride treatment help in blocking endogenous peroxidase acitivity (Kruseman et al (1975) & Brown et al (1976) reduced endogenous staining of red cells by employing a weak (0.001%) concentration of water in the DAB solution.

2. **Background Staining**:

It is due to the uptake of reagents by other tissue components besides the antigen under investigation. These may be connective tissue fibrinogen in blood vessels and immunoglobulin in sera used for staining. Weston & Poole (1973) suggested that paraffin embedded material have increased background staining due to connective tissue whereas over washing in phosphate buffers increases this problem (Gervais 1972) in cryostat sections. The former may be due to the Fc portions of immunoglobulins being attracted to the basic groups present in the collagen fibre or due to IgG in interstitial fluid binding to collagen during formaline paraffin wax processing (Brandtzaeg 1974). This reaction could be avoided by using gelatinized slides.

There are several methods for reducing the background staining. Exposure to highly diluted first antibody i.e. antibody against the antigen in question for 24 to 48 hours at 0°C is

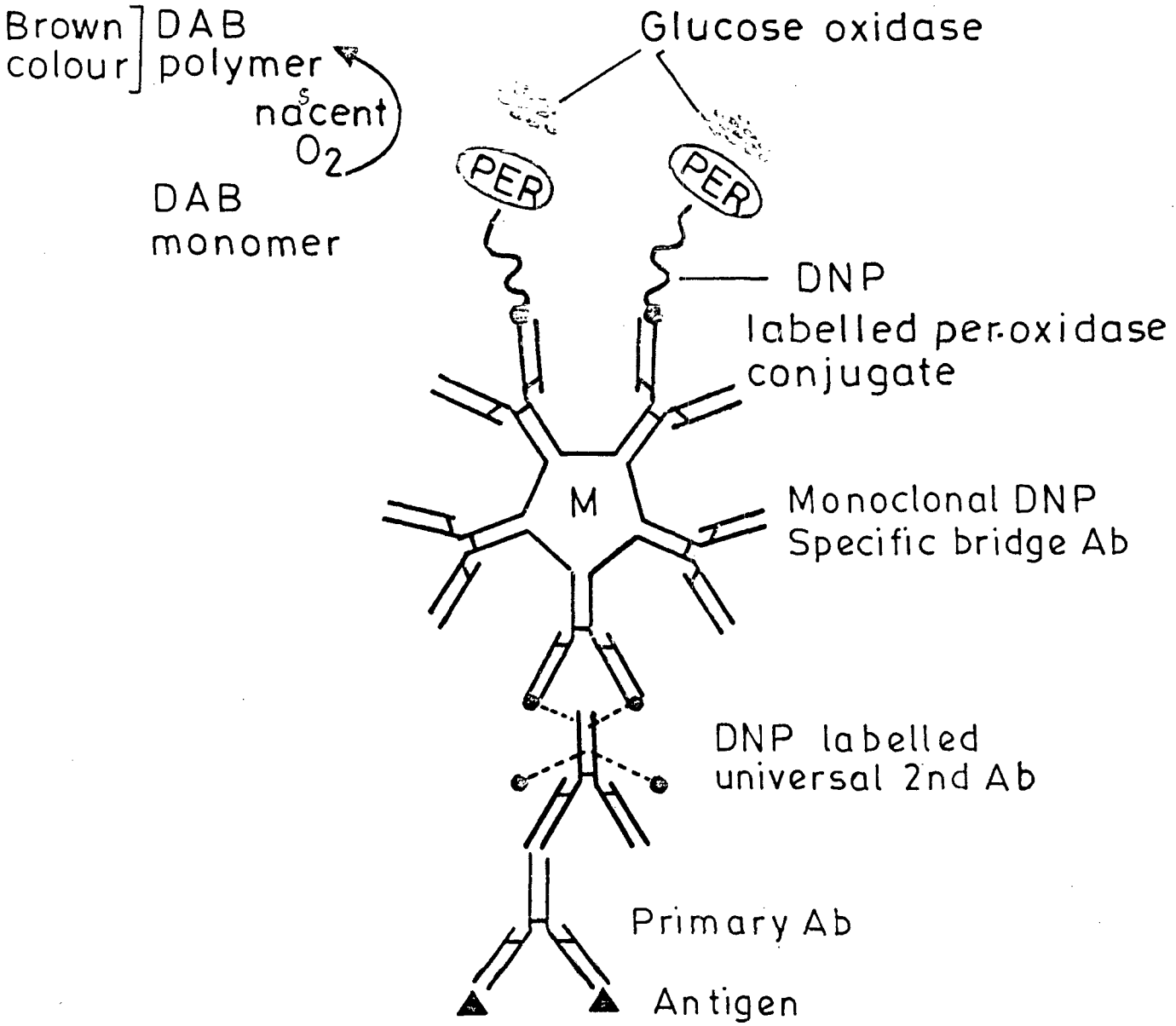

Fig.2.: Schematic representation of DNP-Localisation System-their mode of interaction.

helpful. Further addition of a nonimmune serum for example from the animal species which has been used for producing the secondary antibody reagent or ovalbumin or bovine serum albumin or human blood group-A absorbed swine serum is often employed.

The Dinitrophenyl (DNP) hapten Sandwich Staining (DHSS) Procedure: (Fig.2).

The procedure was discribed by Jasani et al (1981). In this method the primary antibody or the second antibody is labelled with a hapten dinitrophenyl. A monoclonal antihapten antibody is incorporated as a bridge between the primary antibody and the enzyme system used for developing colour. DNP- peroxidase is the enzyme used in this method and DNP glucose oxidase was used to provide nascent oxygen for the DAB reaction (Jasani and William 1985, Jasani et al 1985). This method overcomes the limitation of the previous methods (PAP method). Firstly, the background staining was greatly reduced as highly diluted primary antibody is used. Further, primary antibody was diluted in Bovine serum albumin. Secondly, the selection of unique set of species specific reagents namely anti-immunoglobulin bridge antibody and peroxidase antiperoxidase enzyme marker.

MATERIAL AND METHODS

MATERIALS A ND METHODS

Patients with benign and malignant breast conditions were studied clinically and histologically. Hundred and seven formalin fixed paraffin embedded breast lesions both retrospective and prospective were selected. These included 40 carcinoma 41 fibro-adenomas, 59 benign cystic disease and 8 gynaecomastia. Benign cystic diseases included duct ectasia apocrine metaplasia, lobular hyperplasia and fibroadenosis etc. (Table-4).

TABLE-4

BREAST LESIONS	No.of Cases	Percentage
Carcinoma	40	37.38
Fibroadenoma	41	38.31
Fibroadenosis	8	7.47
Apocrine metaplsia	4	3.73
Duct ectasia	1	0.93
Lactating breast	6	4.67
Gynaecomastia	8	7.47

The technique of Jasani et al (1981) was used through out the present study.

Anterior pituitary collected from post mortem room were taken for positive control studies and lymph node or thyroid

biopsies were taken for negative control. Rabbit antihuman PRL and the secondary detection reagents were obtained from Bio Clin Chemical Ltd. Cardiff, U.K. The colouring reagent was obtained from SIGMA D-5637.

Multiple sections of 5um thickness were cut from formalin fixed paraffin embedded breast tissue as well as anterior pituitary and lymph node tissues blocks. These sections were picked up on gelatenised slides (15% gelatin solution was smeared as the slides and these slides were kept at 40°C overnight for proper setting of gelatin). In each case routine haemotoxylin and eosine staining was carried out along with immunocytochemical method for localization of PRL hormone binding site in several batches. Each batch included a section of pituitary and a section of lymph node or thyroid.

Dewaxing Procedure:

Reagents	Time
Xylol 1	2-4 minutes at RT.
Xylol 2	"
Xylol 3	"
Xylol 4	"
Alcohol 1	"
Alcohol 2	"

Haemotoxylin Eosin Staining Technique:

1. Deparaffinised sections were washed in water.

2. Sections were stained in a solution of hemotoxylin for 2 to 5 minutes.

3. Washed in water till the section is blueing.

4. Decolourised with a solution of acidic alcohol 1% till the cytoplasmic staining by hematoxylin is removed.

5. Washed in running tap water for 5 to 15 minutes.

6. Counterstained in 1% aqueous eosin for 1 minute.

7. Washed rapidly in water and blotted.

8. Dehydrated in several changes of absolute alcohol.

9. Cleared in Xylol and mounted in Canada Balsam.

Immunocytochemical Staining Technique:

1. **Inhibition of Endogenous Peroxidase:**

For this purpose pre-incubation of sections was done in absolute alcohol containing hydrogen peroxidase. The slides were immersed into a mixture of 47.2ml of absolute ethanol and 0.8ml of 33% hydrogen peroxidase for 30 minute at room temperature (Streefkerk 1972).

2. **Rehydration:**

Slides were washed by immersing these into distilled water for 2 minute x 3 changes.

Slides washed by immersing in phosphate buffered saline (PBS), pH = 7.1 (0.01M) for 2 minute x 3 changes.

After this (DNP) dinitrophenyl localization system was used for detection of primary antigenic sites. It represent a

novel, versatile, indirect immunoperoxidase bridge technique designed for the detection unlabelled primary rabbit or mouse antibodies reacted with Ag present in paraffin section.

3. **Incubation with primary antibody (Rabbit antihuman PRL):**

The primary antibody was applied at the dilution of 1:150,00. (Dilution profile experiment was carried out to evaluate the appropriate dilution. The effectiveness of a-PRL was checked by double immunodiffusion against serum of pregnant or lactating women). The dilution was done either in Bovime serum albumin or normal rabbit serum. The slides were covered with this antibody and left overnight incubated at 4°C in a closed moistened box.

4. Washing in phosphate buffer saline was conducted by total immersion of the slides in a coplin jar (1 min x 3 changes).

5. Incubation with second antibody labelled with DNP was for 30 minutes i.e. antibrabbit Ig G labelled with DNP.

6. Washing in PBS (1 minute x 3 changes).

7. Incubation in Ig M anti-DNP bridge antibody (50 to 100ul was done for 30 minutes.

8. Washing in PBS (1 minute x 3 changes).

9. Incubation in DNP peroxidase conjugate for 30 minute (50 to 100ul).

10. Washing in PBS (1 minute x 3 changes).

11. Incubation in DNP glucose oxidase for generation of nacent oxygen (50ul to 100ul) for 30 minutes.

12. Washing in PBS (1 minute x changes).

13. Incubation in diamino benzidine (DAB) + glucose + PBS (0.1M) in a coplin jar overnight at room temperature in a dark place.

14. Washing distilled water for 1 minute x 3 changes.

15. Slides were immersed in 1% acetic acid for 1 minute.

16. Counter staining was done as usual i.e. slides were covered with Mayer's Haemtoxylin for 30-45 seconds. Blueing in running tap water was done for 5 minutes. Finally sections were dehydrated and mounted in Canada Balsam and studied under a microscope.

Control Studies:

1. Sections from postmortem anterior pituitary were used as positive control.

2. Sections from lymphnode or thyroid were used as negative control.

3. Control slides included omission of a PRL and its substitution with the approxpriate dilution of BSA.

Using above method, staining was done in batch including 6-8 breast slides lesion and control slides.

RESULTS

R E S U L T S

One hundred and seven formalin fixed paraffin embedded breast tissue sections were stained by immunocytochemical method for localisation of PRL hormone receptor sites in benign and malignant breast lesion. All malignant lesions were carcinomas

TABLE-5

IMMUNOCYTOCHEMICAL REACTIONS IN BREAST LESIONS

Sl. No.	BREAST LESIONS	IMMUNOCYTOCHEMICAL FINDINGS			
		Positive		Negative	
		No.	%	No.	%
1.	Carcinoma	33/40	(82.5)	7/40	(17.5)
2.	Benign breast disease	34/59	(57.6)	25/59	(42.4)
	Fibroadenoma	23/42	(57)	25/41	(42.4)
	Lactating breast	5/5	(100)	-	-
	Apocrine metaplasia	4/4	(100)	-	-
	Fibroadenosis	3/8	(37.5)	5/8	(62.5)
	Dust ectasia	1/1	(100)	-	-
3.	Gynaecomastia	1/8	(12.5)	7/8	(87.3)

of various histological grades. Majority (82.5%) of the breast carcinoma sections (33/40) revealed positive staining of variable intensity (Fig.3). Amongst the benign lesions majority were

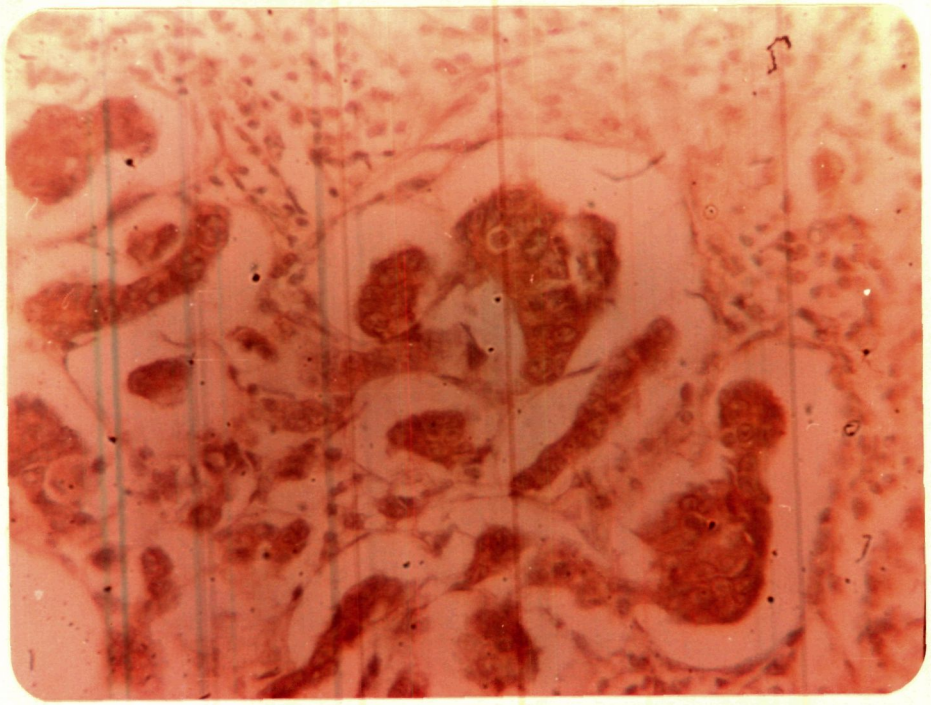

Fig.3: Infiltrating carcinoma breast showing positive staining for PRL of variable intensity (Immunocyto-chemistry x 350).

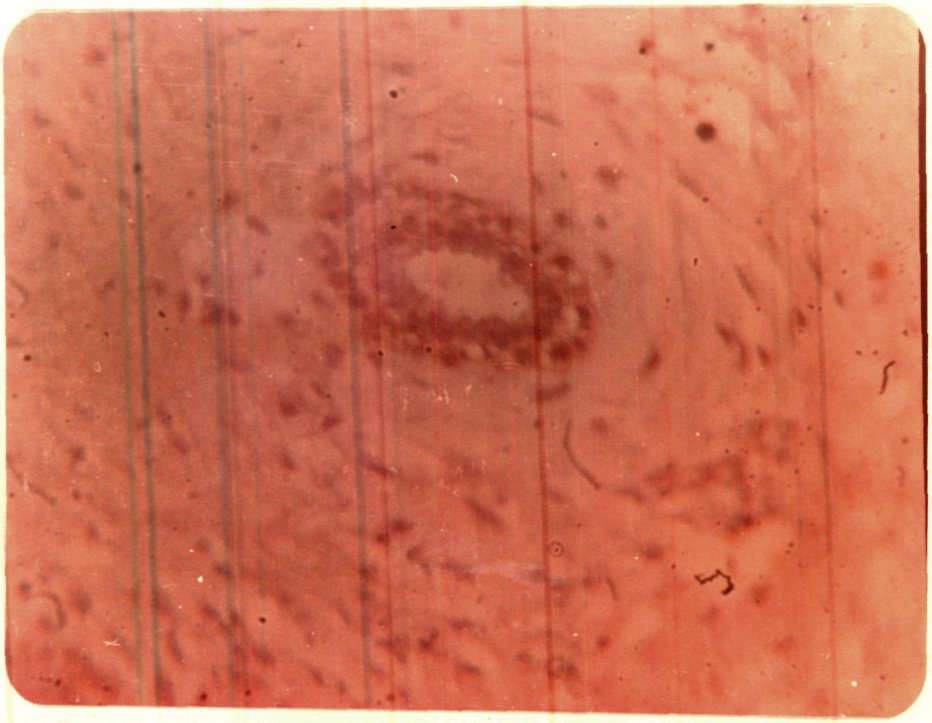

Fig.4.: Benigh Breast Tissue section- Epithelial cell lining duct showing positive reaction for PRL (Immunocyto-chemistry x 350).

fibroadenomas of which 57% of cases (23/41) showed positive staining. Sections of other benign diseases of breast such as fibroadenosis, duct ectasia, gynaecomastia were also studied (Table-5).

Further, it was observed that there was a definite relationship between the age of the patient and intensity of positive immuno-cytochemical staining reaction. Majority of the breast carcinoma slides showed positive reaction in tumours removed from ladies

TABLE-6

RELATIONSHIP OF IMMUNOCYTOCHEMICAL REACTION WITH AGE OF THE PATIENTS

LESIONS	AGE GROU (in years)	POSITIVE		NEGATIVE	
		No.	%	No.	%
Carcinoma	< 30	2/3	(66.66)	1/3	(33.33)
	31-40	6/9	(66.66)	3/9	(33.33)
	41-50	14/16	(92.5)	2/16	(7.5)
	51-60	5/6	(83.3)	1/6	(16.7)
	> 60	6/6	(100)	-	-
Fibroednoma	< 30	10/21	(47.61)	11/21	(52.39)
	31-41	12/17	(70.58)	5/17	(29.42)
	41-50	1/3	(33.33)	2/3	(66.66)

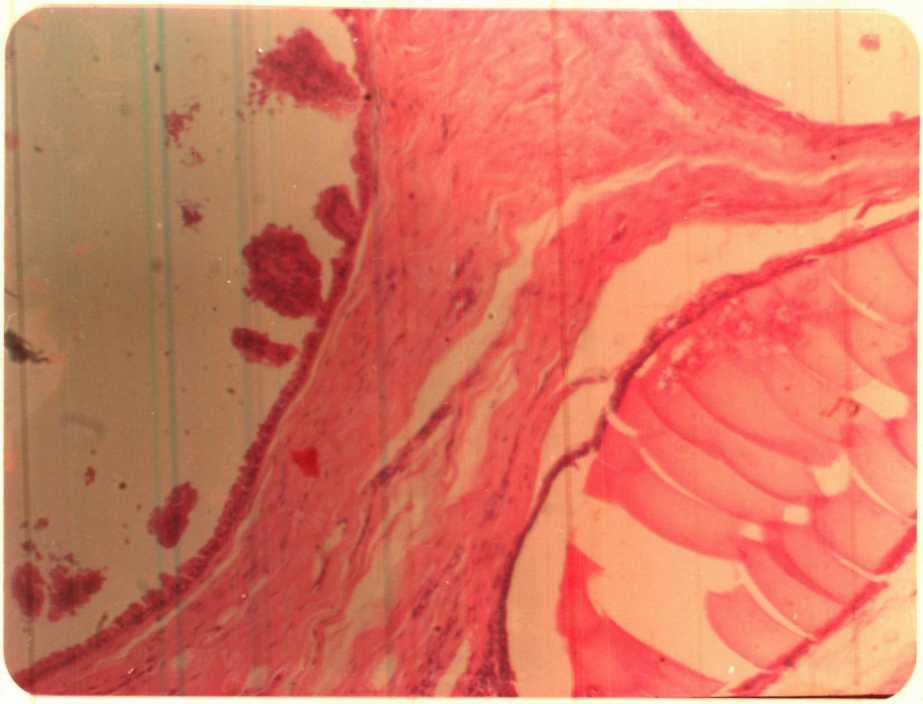

Fig.5.: Section of breast showing cyst lined by apocrine metaplasia (H.E. x 350).

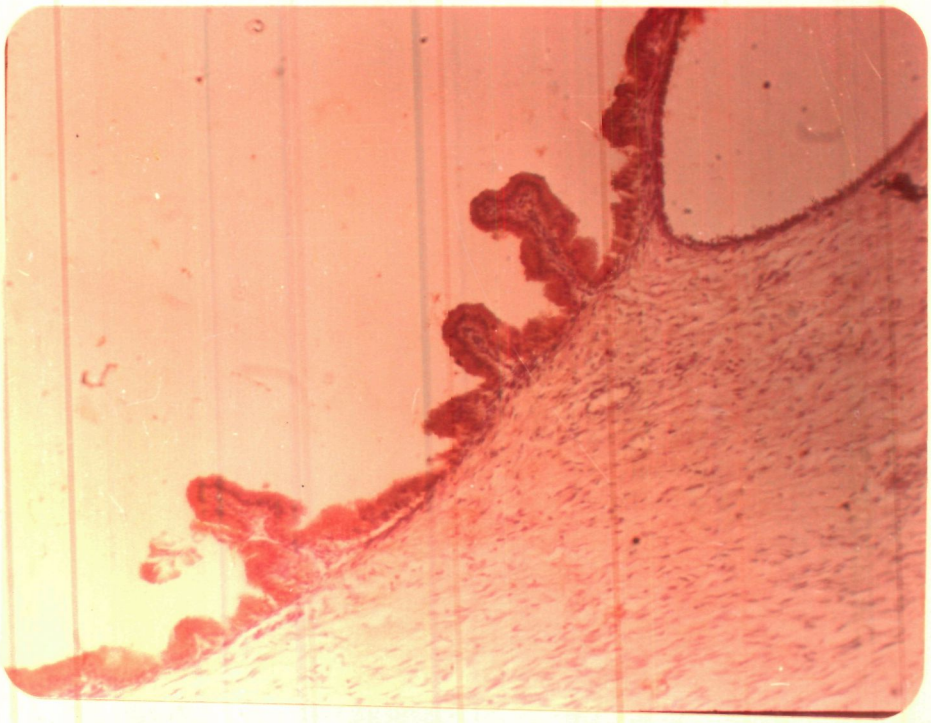

Fig.6.: Intense positive staining reaction for PRL of the above section (Immunocytochemistry x 350).

between the age of 41 to 60 years i.e. in the periminopausal patients whereas the tumours removed from ladies in younger age i.e. during the active reproductive phase of life, the intensity of staining reaction was poor or irregular (Table-6).

Epithelial cell lining of both ducts and acini showed positive staining reaction (Fig.4). The distribution of reaction varied in different individual cases. In some the staining was consistent in all ducts and lobule throughout the section examined whereas in others there was a variation in intensity of staining reaction between ducts and then associated lobules. Amongst the benign cystic disease of the breast the cyst lined by apocrine metaplastic lesions showing papilliomatosis and epitheliosis were strongly positive (Fig. 5 & 6) whereas other benign lesions such as duct ectasia, adenosis, fibroadenosis were weakly or heterogenously positive.

Gynaecomastia were mostly negatively stained. Only 1 out of 8 sections was weakly positive.

Background staining of the breast parenchyma and collagen was not prominent in the majority of the cases though weak nonspecific staining at the periphery of the sections was a commonly observed phenomenon and was ruled out as an edge or a drying artefact.

Positive reaction for PRL was seen in all instances with sections of anterior pituitary but there was no evidence of PRL

binding in lymph node or thyroid section. These two tissues thus acted as positive and negative controls respectively in the present study. Out of total number of forty cases of carcinoma breast only six (15%) cases did not show any immunocytochemical reaction in sections. Rest of the cases revealed a variable reaction, varying from weakly positive to strongly positive.

TABLE- 7

IMMUNOCYTOCHEMICAL REACTION IN DIFFERENT HISTOLOGICAL TYPE OF BREAST CARCINOMA

DIFFERENT HISTOLOGICAL TYPES OF CARCINOMAS	NO.OF CASES	IMMUNOCYTOCHEMICAL REACTION			
		Weak	Moderate	Strong	Negative
Wall differentiated	19	7(36.8)	5(26.3)	6(31.5)	1(.5)
Poorly differentiated	2	-	1(50%)	1(50%)	-
Papillary adenocarcinoma	3	1(33)	-	-	2(66%)
Lobular carcinoma	6	1(16.66%)	2(33.33%)	-	3(50%)
Carcinoma associated with Paget's disease	2	2(100)	-	-	-
Metastising into lymph node	8	-	5(62.5%)	3(38%)	-
TOTAL	40	11(27.5%)	13(32.5%)	10(25%)	6(15%)

On further analysis of grade of malignancy in relation to intensity of immunocytochemical staining revealed that the

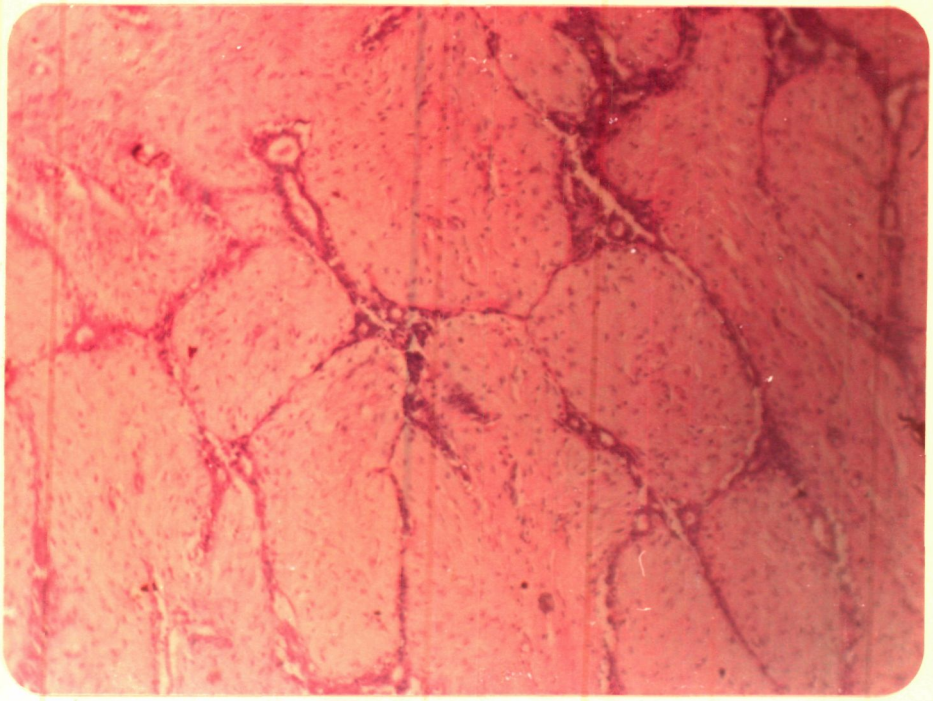

Fig.8.: Section of fibroadenoma (H.E. x 350).

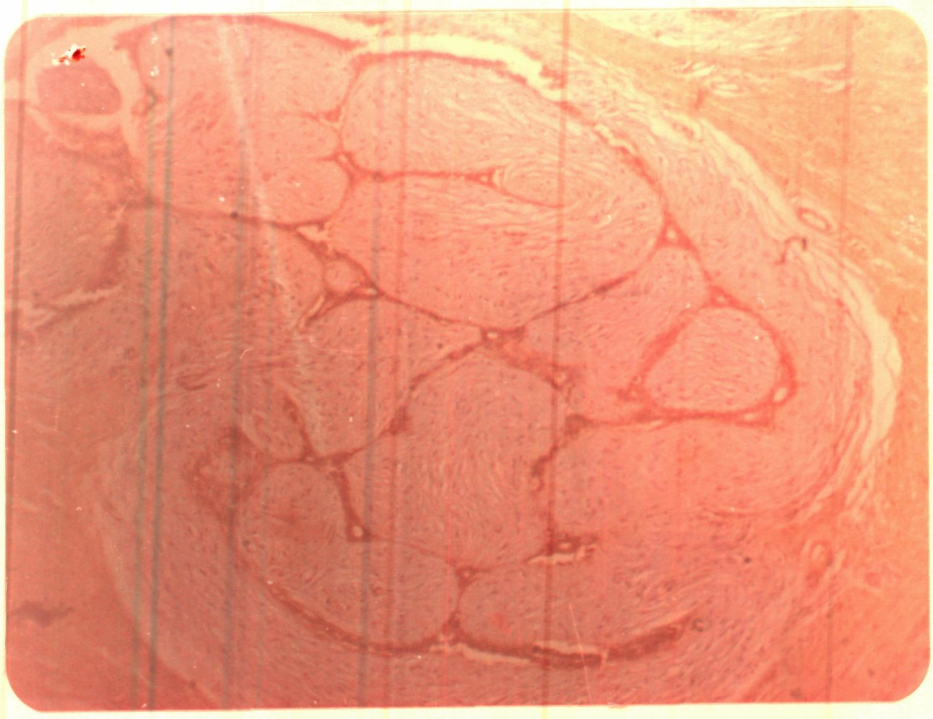

Fig.9.: Positive staining reaction for PRL of the above section (Immunocytochemistry x 350).

pituitary sections and positively stained breast tissue studies whereas concentrated a-PRL increases background staining. The optimal staining reaction was obtained at the dilution of 1:150,00 of a PRL diluted in BSA.

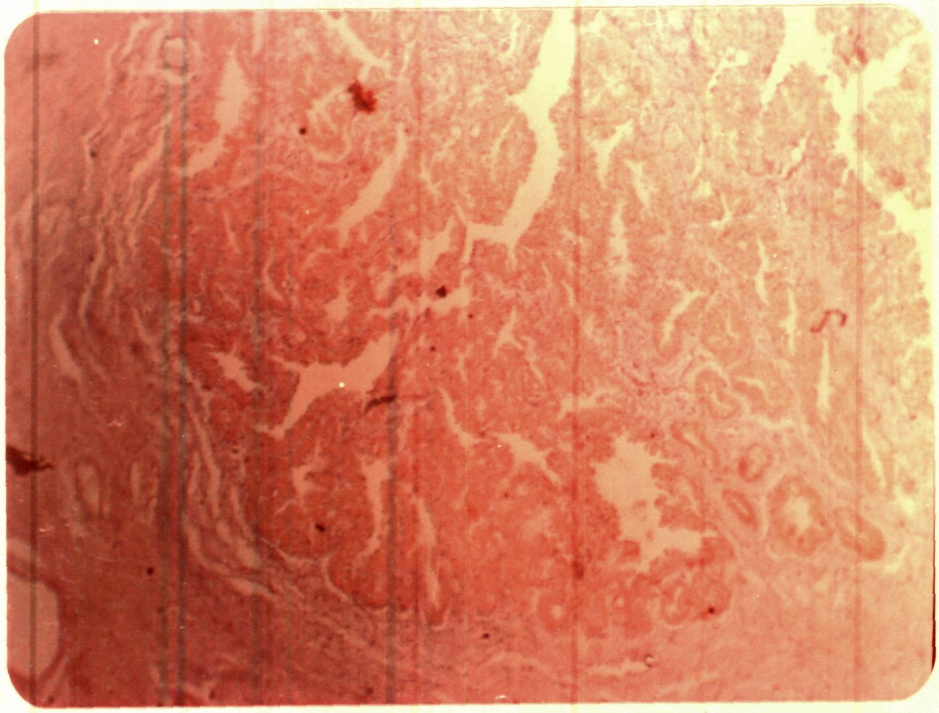

Fig.10: Lobular hyperplasia showing positive reaction for PRL (Immunocytochemistry x 350).

DISCUSSION

DISCUSSION

This study has indicated fairly consistant presence of immunohistochemical binding of PRL in both malignant and benign breast tissues. Sections of more than 5 years old paraffin blocks of formalin fixed, breast tissues could be stained as we have employed a novel and a highly sensitive method of immunohistochemically localising the PRL binding sites. The application of immunohistochemistry to the detection of PRL binding sites in human breast tumours is potentially of great interest. Indeed a tumour may consists of a mixture of positive and negative cells. Since the radioreceptor assay requires tissue homogenization, this technique is not able to distinguish between positive and negative cells. On the contrary, this is theoretically possible by immunocytochemistry.

De-Souza et al (1976) were the first to claim that PRL-R could be visualized with the immuno-peroxidase technique. Then Dhadly and Walker (1983) using purified human prolactin and a specific antiserum to human PRL, following peroxidase anti-peroxidase method, were able to detect PRL binding in benign and malignant human breast tissue. All these workers have used fresh frozen material and found this to be essential for immunohistological staining. In this present study, PRL binding sites have been detected by immunohistochemistry in human benign breast tissue and breast carcinomas. Our success with formalin fixed, paraffin embedded tissue is contrary to these

previous reports, may be because we have followed a novel and a sensitive method of immunoperoxidase labelling. Earlier Purnell et al (1982) were able to demonstrate cytoplasmic PRL binding in paraffin embedded tissue but had used antiserum against bovine PRL while we have used anti-serum against human PRL.

The dilution of anti-PRL required for staining was 100 times greater in the present study as compared to others (Salih et al 1972). The second antibody heavily substituted with hapten have proved effective in localising hormones binding sites, the number of which in tissue may be very low. The hapten dinitrophenyl (DNP) reacts efficiently with immunoglobulins without altering its capacity to bind antigen. Further use of monoclonal IgM type of immunoglobulin as bridge antibody results in higher staining sensitivity. The dilution fraction can be increased and thus small amount of primary antibody is required.

Background staining i.e. uptake of the immunoreagents by other tissue components besides the antigen under investigation makes it difficult to interpret the results of the immunoperoxidase technique. There are several methods for reducing this back ground staining (Ternyanck 1976, Reading 1977). We have followed the method of Burns (1975b) in which addition of bovine serum albumin reduces background staining. Further exposure to highly diluted anti-PRL overnight at 4°C is helpful in reducing the background staining.

In the present work while studying the immunohistochemical reaction in the breast tissue it was observed that the breast tissue responds to the staining reaction differently. Meaning thereby that while multiple sections from the same breast were examined, some part of the tissue including the normal gave positive reaction, while other parts were negative. Not only that even the intensity of staining was variable in the same tissue. This type of heterogene ity in the staining reaction was constantly seen in all the lesions including benign, malignants and non-neoplastic. Same has been the experience of other workers (Purnell et al 1982, Dhadly & Walker 1983).

A higher incidence (82.5%) of PRL binding in breast carcinoma was observed in the present study as compared to the previous ones (56%). (Purunell et al, 1982, Dhadly and Walker 1983), may be due to more sensitive technique that we have used throughout this study. The result of PRL obtained by us may be encouraging and this may prove an useful method for screening PRL- growth hormone dependent breast tumours PRL is one of the principal hormones regulating the alveolar function of the mammary gland (Anerson R.R. 1974). The role of PRL is implicated in mammary tumorigenesis of experimental animals (Welsch C.W. and Nagasawa H. 1977). Further more, there is growing evidence that PRL may also be involved in the development and progression of some human breast cancers (Wilson et al, 1980, Teyssot et al 1981, Shiu P.C. and Iwascow B.M. 1985). The PRL dependency of mammary carcinoma

in women is not yet well understood as the interpretation of plasma PRL levels are difficult because of the marked variation and pulsatility of its secretion.

The importance of PRL in human breast cancer is stressed as women at increased risk of the disease because of a late age first baby, late menopause, nulltiparity, obesity, or a family history of breast cancer have raised blood levels of the hormones (Kwa et al, 1976, 1978, 1981, Wang et al 1986). We have found immunocytochemically that majority of the breast carcinoma slides show positive reaction of the patients above the age of 41 years (Table-6). The findings of PRL presence in greater proportion in perimenopausal breast cancer tissue is of significance as this is in consonance with the presence of oestrogen receptor in breast cancer. Sherman & Korenman (1974) have hypothesized that unopposed oestrogenic action in the perimenopausal period (Say 0 to 8 bears before menopause) due to defective progesterone production is a key determinant of subsequent risk. The unopposed oestrogen is due to defective luteal phases as the menopause approaches. Kwa et al (1978) observed that post menopausal patients who subsequently develop breast cancer have raised plasma PRL levels upto 5 years before the clinical diagnosis of disease is made. This could suggest incidence of higher positivity of PRL binding of peri- and post menopausal patients in our study. However Partridge and Hannel (1979) who examined nine cases of human breast

carcinoma have found that specific binding of PRL was occuring in at least three of the nine specimen examined. These "prolactin receptor positive" tumours were all from premenopausal patients. Thus no definite opinion could be made out with regard to menopausal status.

Earlier the decision as to whether endocrine treatment is likely to benifit patients with metastatic breast cancer was most frequently made on the basis of estimation of estrogen receptors in the tumours. However, this dependency of carcinoma on oestrogen receptors seems incomplete, as lack of oestrogen receptors indicates little likelihood of beneficial effects from endocrine treatment. Also the patients with an oestrogen receptor positive tumour has only a 55-60% chance of benefitting from endocrine therapy. Thus recent research indicates the significance of additional factors such as different hormones in estimating more accurately the tumours endocrine responsiveness stressed the importance of the search for PRL's role in human breast cancer. Since oestrogens are well known to stimulate PRL secretion (Shull & Gorshi 1984, William et al 1985) it could be stated that oestrogen and PRL may interact in promoting mammary tumorigenesis (Ben-David 1980). Ben David (1981) in their further studies on binding sites for PRL (PRL-R) reported a higher dependency of breast cancer on PRL than on steroid receptors.

The multiple hormonal interactions that take place in the normal growth and development of the breast has been reviewed by Topper and Freeman (1980). It would, therefore, be illogical, at least theoretically, to implicate any single hormonal abnormality as a prime factor in the aetiology of breast disease. Recently women with BBD have shown to exhibits an increased PRL response to TRH or doperidone provocation tests (Kumar et al, 1984). PRL binding was detected in 57.6% of BBD, similar to that obtained by Kumar et al (1987). These findings are contradictory to the observation made by Dhadly and Walker (1983) who reported positive reaction in all the benign breast lesions. However, no staining was obtained in any of the benign breast lesions by De-Souza et al 1979). Carlo et al (1984) also reported presence and biochemical characterization of PRL-R in human benign breast tumours.

The experience of various workers varied in the field of immunocytochemical staining of sections of fibroadenomas. In the present study in a good percentage of cases (57) there was positive reaction whereas other workers could not demonstrate the PRL binding sites. It was because perhaps we used a modified, more sensitive technique for demonstration of the receptors. The role of PRL in the overall causation of benign breast pathology is uncertain. The determination and the level of specific PRL binding sites in abnormal breast tissue may therefore be useful in better understanding of the pathophysiology of benign breast disease.

In the present study the slides showing apocrine metaplasia exhibited intense staining. This fact is also shown by Kumar et al (1987). He further stated that addition of exogenous PRL onto the sections led to considerable selective enhancement of anti PRL mediated staining of the apocrine metaplasia cells which may indicate that the cells are involved in the production of PRL- specific binding sites, probably PRL receptors. If so, this could suggest a target function on the part of metaplasic cells for PRL. Further apocrine metaplasia or pink cell change, characterised by high cylindrical cells with granular eosinophilic cytoplasm projecting as 'Snouts' into the lumen of the ducts and cysts has long been regarded of no significance in cancer risk. Similarly, breast carcinoma originating from apocrine metaplasia was considered very rare by Footi & Steward (1945). On the other hand, Page et al in 1978 reported that patients whose initial biopsy have shown apocrine metaplasia may have an increased cancer risk. Later on Haagenson (1986) also supported this observation. He also observed quite higher incidence of apocrine type of breast carcinoma, probably arising in metaplastic breast tissue. It is possible that the PRL positive breast cancer cells seen in the present study may represent cells depicting apocrine type breast carcinoma.

It is interesting to note that the histopathological differentiation of breast tumours has got direct relationship with the presence of PRL- binding receptors in the study as it was

observed that well differentiated tumours has less intense reaction to immunocytochemical stain, as opposed to that of Dhadly and Walker (1983) who reported the presence of more PRL binding sites in well differentiated carcinoma. Also in our study metastasing tumours showed stronger reaction to PRL immunocytochemical staining. This observation is in correlation with the work of Wang et al (1986) in which they identified subgroups of patients in which either preoperative and/or postoperative PRL levels were significantly correlates to survival in all cases, the least favourable prognosis was associated with the highest PRL levels. This general trend has been confirmed by Dowsell et al (1987) in advanced disease as well as in early disease (Dowselt et al 1987) and also by Holtkamp et al (1984). However Bonneterre et al (1987) while studying the prognostic significance of PRL receptors in human breast cancer have stated that relapse free survival was higher in patients with node metastasis. Thus no definite conclusion could be made with regard to PRL incidence and metastasing tumours. The hypothesis that PRL might support the growth of breast cancer in vivo is indeed supported by invitro experiments, as shown by Peyrat et al (1982) and also by other workers (Carto et al 1979, Partridge et al, 1979). These workers have reported that some human breast tumours contain low but measurable levels of free PRL receptors and this low level of receptors in human breast cancer biopsies

does not rule out an important role of PRL in the development of tumour.

All these indicate that PRL might be significant in the support of breast cancer growth in vivo and/or conversely that hyperprolactinaemia might be only a consequence, by an unknown mechanism of the progression of the disease. It may be stated that either an unknown factor is secreted which acts at the hypothalmo pituitary level to enhance PRL secretion, or perhaps that the tumour itself secretes PRL.

C O N C L U S I O N S

CONCLUSION

From this study following conclusion were drawn.

PRL binding sites could be detected in human breast tissue by immunocytochemistry. Formalin fixed paraffin embedded, upto five years or more old breast blocks tissue section have shown satisfactory staining for PRL binding site. Both benign and malignant breast tissue slices have shown variable degree of positive staining for PRL binding site. The breast carcinoma tissue slices have shown more consistent immunostain as compared to fibroadenoma tissue. Since the PRL binding sites were more commonly seen in carcinoma breast tissue one can not exclude the possibility of the role of PRL in mammary tumorigenesis. Further finding of PRL binding sites presence in greater proportion in perimenopausal breast cancer patients is of significance, since this is in consonance with the presence of oestrogen receptor in breast cancer.

Apocrine change in epithelial lining had specific PRL binding sites. This finding may suggest a role of PRL in the aetiology of breast cancer through the induction of apocrine change since it has recently been shown to be associated with an increased risk of breast cancer.

In view of these findings, staining with PRL antibody

may prove to be a useful marker for determining of high risk benign breast disease biopsies and immunohistochemical staining for PRL may be useful in identifying apocrine type of breast carcinoma.

B I B L I O G R A P H Y

B I B L I O G R A P H Y

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